

In re application of  
Gregory M. Fahy  
Application No.: 09/916,396  
Page 2 of 15

Atty. Dkt. No. CentMed.020A  
(074066-0104)

### Amendments to the Claims/Listing of Claims

Please amend claims 1, 3, 4, 6, 7, 10, 13, 15, 22, 25-30, 32 and 36, add new claims 38-46, and cancel claims 8, 9, 19-21, 31, 33-35 and 37 as follows. This listing of claims will replace all prior versions, and listings of claims in the present application.

1. (Currently amended) In a carrier solution compatible with the antinucleating effects of polyglycerol, polyvinyl alcohol, or a copolymer of vinyl alcohol and vinyl acetate in the presence of vitrifiable concentrations of cryoprotectants, wherein said solution is capable of being used for the introduction and washout of vitrifiable concentrations of cryoprotectants in a viable cell, tissue or organ, the improvement comprising inclusion of mannitol and lactose in the solution.

2. (Previously presented) The solution of Claim 1 further comprising vitrifiable concentrations of cryoprotectant.

3. (Currently amended) ~~The~~ In a solution of Claim 2 for the introduction and washout of vitrifiable concentrations of cryoprotectants in a viable cell, tissue or organ, the improvement comprising inclusion of mannitol, lactose and vitrifiable concentrations of cryoprotectant in the solution, wherein said cryoprotectant comprises dimethyl sulfoxide, formamide, and ethylene glycol.

4. (Currently amended) ~~The~~ In a solution of Claim 2 for the introduction and washout of vitrifiable concentrations of cryoprotectants in a viable cell, tissue or organ, the improvement comprising inclusion of mannitol, lactose and vitrifiable concentrations of cryoprotectant in the solution, wherein said cryoprotectant comprises polyvinyl alcohol, or a copolymer of vinyl alcohol and vinyl acetate.

5. (Cancelled)

In re application of  
Gregory M. Fahy  
Application No.: 09/916,396  
Page 3 of 15

Atty. Dkt. No. CentMed.020A  
(074066-0104)

6. (Currently amended) ~~The~~ In a solution of Claim 2 for the introduction and washout of vitrifiable concentrations of cryoprotectants in a viable cell, tissue or organ, the improvement comprising inclusion of mannitol, lactose and vitrifiable concentrations of cryoprotectant in the solution, wherein said cryoprotectant comprises one or more of polyglycerol and polyvinylpyrrolidone.

7. (Currently amended) The solution of Claim 2 wherein said solution further comprises polymers selected from the group consisting of: polyglycerol, polyvinylpyrrolidone, polyvinyl alcohol, and a copolymer of vinyl alcohol and vinyl acetate ~~and sucrose; and~~  
~~wherein said solution has a tonicity of 1.2 to 2.0 times that of a solution that does not cause osmotic volume changes of said cell, tissue or organ.~~

8-9. (Cancelled)

10. (Currently amended) ~~The~~ In a carrier solution of Claim 1, further comprising compatible with the antinucleating effects of polyglycerol, polyvinyl alcohol, or a copolymer of vinyl alcohol and vinyl acetate in the presence of vitrifiable concentrations of cryoprotectants, wherein said solution is capable of being used for the introduction and washout of vitrifiable concentrations of cryoprotectants in a viable cell, tissue or organ, the improvement comprising inclusion of mannitol, lactose and glucose in the solution.

11-12. (Cancelled)

13. (Currently amended) ~~The~~ A carrier solution of Claim 10 further comprising, compatible with the antinucleating effects of polyglycerol, polyvinyl alcohol, or a copolymer of vinyl alcohol and vinyl acetate in the presence of vitrifiable concentrations of cryoprotectants, wherein said solution is capable of being used for the introduction and washout of vitrifiable concentrations of cryoprotectants in a viable cell, tissue or organ, said solution comprising: 45 mM mannitol, 45 mM lactose, 7.2 mM potassium phosphate, 1

In re application of  
Gregory M. Fahy  
Application No.: 09/916,396  
Page 4 of 15

Atty. Dkt. No. CentMed.020A  
(074066-0104)

~~mM calcium chloride, 2 mM magnesium chloride,~~ 5 mM reduced glutathione, 28.2 mM potassium chloride, 10 mM sodium bicarbonate, and 1 mM adenine HCl.

14. (Cancelled)

15. (Currently amended) The solution of Claim ~~[[10]]~~ 7, wherein said solution has a tonicity of between 1.1 to 2.0 times that of a solution that does not cause osmotic volume changes of said cell, tissue or organ.

16-21. (Cancelled)

22. (Currently amended) ~~The~~ In a carrier solution of Claim 1 compatible with the antinucleating effects of polyglycerol, polyvinyl alcohol, or a copolymer of vinyl alcohol and vinyl acetate in the presence of vitrifiable concentrations of cryoprotectants, wherein said solution is capable of being used for the introduction and washout of vitrifiable concentrations of cryoprotectants in a viable cell, tissue or organ, the improvement comprising inclusion of mannitol and lactose in the solution, wherein said solution has a tonicity of between 1.1 to 2.0 times that of a solution that does not cause osmotic volume changes of said cell, tissue or organ.

23-24. (Cancelled)

25. (Currently amended) ~~The~~ In a carrier solution of Claim 1 for the introduction and washout of vitrifiable concentrations of cryoprotectants in a viable cell, tissue or organ, the improvement comprising inclusion of mannitol and lactose in the solution, wherein the lactose and mannitol are each present at 45 mM.

26. (Currently amended) ~~The~~ In a carrier solution of Claim 1 for the introduction and washout of vitrifiable concentrations of cryoprotectants in a viable cell, tissue or organ,

In re application of  
Gregory M. Fahy  
Application No.: 09/916,396  
Page 5 of 15

Atty. Dkt. No. CentMed.020A  
(074066-0104)

the improvement comprising inclusion of mannitol and lactose in the solution, further comprising 10 mM bicarbonate.

27. (Currently amended) The solution of claim 2, ~~further comprising said solution comprising:~~ 22.305% w/v dimethyl sulfoxide, 12.858% w/v formamide, 23.837% w/v ethylene glycol, and 1% w/v ~~X1000 polyvinyl alcohol or a copolymer of vinyl alcohol and vinyl acetate, and~~ 4% w/v decaglycerol ~~in LM5 carrier~~.

28. (Currently amended) The solution of claim 2, ~~further comprising said solution comprising:~~ 22.305% w/v dimethyl sulfoxide, 12.858% w/v formamide, 16.837% w/v ethylene glycol, ~~and~~ 1% w/v ~~X1000 polyvinyl alcohol or a copolymer of vinyl alcohol and vinyl acetate,~~ 4% w/v decaglycerol, and 7% w/v acetol ~~in LM5 carrier~~.

29. (Currently amended) The solution of claim 2, ~~further comprising said solution comprising:~~ 22.305% w/v DMSO, 12.858% w/v formamide, 16.837% w/v ethylene glycol, 1% w/v ~~X1000 polyvinyl alcohol or a copolymer of vinyl alcohol and vinyl acetate,~~ 1% w/v decaglycerol, and 7% w/v polyvinylpyrrolidone ~~5000 in LM5 carrier~~.

30. (Currently amended) The solution of Claim 7, said solution having a tonicity of 1.2 to ~~[[1.5]]~~ 2.0 times that of a solution that does not cause osmotic volume changes of said cell, tissue or organ.

31. (Cancelled)

32. (Currently amended) The solution of claim ~~[[31]]~~ 13 further comprising 1 mM  $\text{CaCl}_2$  and 2 mM  $\text{MgCl}_2$ .

33-35. (Cancelled)

In re application of  
Gregory M. Fahy  
Application No.: 09/916,396  
Page 6 of 15

Atty. Dkt. No. CentMed.020A  
(074066-0104)

36. (Currently amended) The solution of claim ~~19, further comprising PVP 5000~~ 7, wherein said polymer is polyvinylpyrrolidone.

37. (Cancelled)

38. (New) A carrier solution compatible with the use of polyglycerol, polyvinyl alcohol, or a copolymer of vinyl alcohol and vinyl acetate, as an antinucleator in the presence of vitrifiable concentrations of cryoprotectants, wherein said carrier solution is capable of being used for the introduction and removal of vitrifiable concentrations of cryoprotectant in a viable cell, tissue, or organ, said solution comprising glucose, lactose, mannitol, potassium phosphate, potassium chloride, sodium bicarbonate, reduced glutathione, and adenine.

39. (New) The carrier solution of Claim 38, further comprising one or more of polyglycerol, polyvinyl alcohol or a copolymer of vinyl alcohol and vinyl acetate, polyvinylpyrrolidone, dimethyl sulfoxide, formamide, and ethylene glycol.

40. (New) The solution of Claim 39, wherein polyglycerol is present at 0.5-4% w/v, polyvinyl alcohol or a copolymer of vinyl alcohol and vinyl acetate is present at 0.1-1% w/v, polyvinylpyrrolidone is present at 0-7% w/v, dimethyl sulfoxide is present at 20.926-24.208% w/v, formamide is present at 12.858-17.234% w/v, ethylene glycol is present at 15.919-23.837% w/v, and further comprising acetol at 0-7% w/v.

41. (New) A carrier solution compatible with the use of polyglycerol, polyvinyl alcohol, or a copolymer of vinyl alcohol and vinyl acetate, as an antinucleator in the presence of vitrifiable concentrations of cryoprotectants, wherein said carrier solution is capable of being used for the introduction and removal of vitrifiable concentrations of cryoprotectant in a viable cell, tissue, or organ, said solution comprising 90 mM glucose, 45 mM lactose, 45 mM mannitol, 7.2 mM potassium phosphate, 28.2 mM potassium chloride, 10 mM sodium bicarbonate, 5 mM reduced glutathione, and 1 mM adenine.

In re application of  
Gregory M. Fahy  
Application No.: 09/916,396  
Page 7 of 15

Atty. Dkt. No. CentMed.020A  
(074066-0104)

42. (New) The carrier solution of Claim 41, further comprising polyglycerol, polyvinyl alcohol or a copolymer of vinyl alcohol and vinyl acetate, polyvinylpyrrolidone, dimethyl sulfoxide, formamide, and ethylene glycol.

43. (New) The solution of Claim 42, wherein polyglycerol is present at 0.5-4% w/v, polyvinyl alcohol or a copolymer of vinyl alcohol and vinyl acetate is present at 0.1-1% w/v, polyvinylpyrrolidone is present at 0-7% w/v, dimethyl sulfoxide is present at 20.926-24.208% w/v, formamide is present at 12.858-17.234% w/v, ethylene glycol is present at 15.919-23.837% w/v, and further comprising acetol at 0-7% w/v.

44. (New) The solution of Claim 1, further comprising polyglycerol at 0.5-4% w/v, polyvinyl alcohol or a copolymer of vinyl alcohol and vinyl acetate at 0.1-1% w/v, polyvinylpyrrolidone at 0-7% w/v, dimethyl sulfoxide at 20.926-24.208% w/v, formamide at 12.858-17.234% w/v, ethylene glycol at 15.919-23.837% w/v, and acetol at 0-7% w/v.

45. (New) The solution of Claim 1, in which all components are concentrated 5-fold.

46. (New) A cryoprotectant solution comprising a carrier solution compatible with the antinucleating effects of polyglycerol, polyvinyl alcohol, or a copolymer of vinyl alcohol and vinyl acetate in the presence of vitrifiable concentrations of cryoprotectants, said solution comprising mannitol, lactose and vitrifiable concentrations of cryoprotectant.